Group Art Unit: 1636

Serial Number: 10/073,135

AMENDMENTS TO THE SPECIFICATION

Please amend the paragraph starting on page 16, line 9 as follows:

The PACAP gene targeting vector was constructed from genomic DNA clones (λ MPL4 and λ MPL18) (Yamamoto, K., Hashimoto, H., Hagihara, N., Nishino, A., Fujita, T., Matsuda, T. & Baba, A. (1998) Gene 211, 63-69) isolated from a 129/SvJ mouse genomic library. A 2.1-kb PvuII fragment of the PACAP gene containing part of exon 5 and the 3' flanking region was inserted 3' to the neomycin resistant (neo) gene (derived from pGEM7-PGK-neo-polyA) in pBluescript KS(+). A MC1 promoter-driven diphtheria toxin A-fragment (DT) gene (derived from pMC1DTpA) was then inserted 5' to the neo gene. Subsequently, a 5.3-kb HindIII genomic DNA fragment containing exons 1A through 4 was inserted between the DT and neo genes, to generate the PACAP targeting vector (Fig. 1A). The linearized vector was electroporated into 129/Ola mouse-derived E14tg2a ES cells. Targeted clones were identified by Southern blot analysis using external 0.42-kb and 1.1-kb probes and microinjected into C57BL/6 E3.5 blastocysts. Two highly chimeric males showed germ-line transmission and were mated with C57BL/6 wild-type females to produce F1 heterozygous mice. F1 heterozygotes were mated with C57BL/6 mice to produce the F2- and F3-generation mice, which were used in this study unless otherwise specified. The null allele of PACAP was also backcrossed 5 times with ICR mice, whose litter sizes were much larger than C57BL/6 mice. Wild-type mice and mice homozygous for the mutant PACAP gene were

Serial Number: 10/073,135 Group Art Unit: 1636

obtained from the intercross of heterozygous animals, and experiments were conducted with adult (three to five months old) mice. Reverse transcription-polymerase chain reaction (RT-PCR) was performed as described in Hashimoto, H., Hagihara, N., Koga, K., Yamamoto, K., Shintani, N., Tomimoto, S., Mori, W., Koyama, Y., Matsuda, T. & Baba, A. (2000) J. Neurochem. 74, 501-507, using the following PACAP gene exon-specific primers: exon 3, 5'-AGA AGA CGA GGC TTA CGA CCA G-3' (SEQ ID NO:1) (sense); exon 4, 5'-ACG ACC GAC TGC AGG TAC TTC-3' (SEQ ID NO:2) (antisense); and exon 5, 5'-TTT CTT GAC AGC CAT TTG TTT TCG G-3' (SEQ ID NO:3) (antisense). The β -actin housekeeping gene was simultaneously reverse transcribed and amplified as previously described in Kitanaka, J., Hashimoto, H., Sugimoto, Y., Sawada, M., Negishi, M., Suzumura, A., Marunouchi, T., Ichikawa, A. & Baba, A. (1995) Biochim. Biophys. Acta 1265, 220-223. In situ hybridization analysis was performed on parasagittal brain sections as described in Hashimoto, H., Nogi, H., Mori, K., Ohishi, H., Shigemoto, R., Yamamoto, K., Matsuda, T., Mizuno, N., Nagata, S. & Baba, A. (1996). J. Comp. Neurol. 371, 567-577. Two different cDNA fragments of mouse PACAP (Yamamoto, K., Hashimoto, H., Hagihara, N., Nishino, A., Fujita, T., Matsuda, T. & Baba, A. (1998) Gene 211, 63-69), a 431-bp cDNA fragment (-116 to 315, where +1 represents the nucleotide position of the ATG initiation codon) spanning exons 2-4, and a 198-bp fragment (340 to 537) containing part of the exon 5 coding sequence deleted by homologous recombination, were used as templates to synthesize 35S-CTP-labeled cRNA probes. The expression of the

Serial Number: 10/073,135 Group Art Unit: 1636

biologically active mature PACAP isoform, PACAP38, was studied in brain by a radioimmunoassay kit (Peninsula Labs, Belmont, CA, USA).